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Influence of Light Delivery on Photodynamic Synovectomy in an Antigen-Induced Arthritis Model for Rheumatoid Arthritis

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Background and Objective: **Minimally invasive synovectomy techniques have been unsuccessful due to lack of selectivity. The purpose of this study was to evaluate the potential of photodynamic therapy to destroy diseased synovium in an antigen-induced arthritis model.**
Study Design/Materials and Methods: **Three sets of experiments evaluated the biodistribution and treatment effects of Photofrin (PF) in rabbits with bilateral knee antigen-induced arthritis. The first set of experiments evaluated the biodistribution of PF in articular tissues of 30 rabbits from 6–72 hours after systemic injection of 2 mg/kg. In the second series of experiments, light was delivered to the knee joint via cleaved optical fibers, whereas for the third, light was delivered via a 600 μm diffusion tip fiber. Tissues were harvested at 2 and 4 weeks posttreatment.**
Results: **The biodistribution experiments demonstrated maximal uptake in inflamed synovium at 48 hours and a lack of uptake in normal tissues. With bare cleaved fibers, necrosis was observed in one specimen at 2 weeks and was absent in all specimens at 4 weeks. In the third experiment, synovial necrosis was observed in 3 of 7 specimens at 2 weeks and 3 of 8 at 4 weeks. No damage to articular cartilage or periarticular tissues was seen with either mode of light delivery.**
Conclusion: **These studies indicate that selective destruction of synovium can be achieved with PF and suggest that optimization of light delivery techniques will play an important role in development of this new technique. Lasers Surg. Med. 22:147–156, 1998.** **© 1998 Wiley-Liss, Inc.**

Key words: synovectomy; photodynamic therapy; rheumatoid arthritis

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INTRODUCTION

For the patient with active rheumatoid arthritis who fails medical management, surgical synovectomy is the principal mode of treatment [1–4]. Alternatives include chemical and radiation synovectomy. Attempts to destroy proliferative synovium with chemical compounds have been too destructive of normal articular tissues to merit clinical use [5–8]. Radiation synovectomies have been performed successfully; however, longer lived radionuclides are subject to extra-articular leakage [9–12] and limited availability of short lived radioisotopes may preclude widespread use [13,14]. Recently, photodynamic therapy, PDT, has been introduced for the treatment of rheumatoid arthritis [15–17]. In this study, we investigate the feasibility of using PDT to destroy diseased synovium in an effort to develop a less invasive and less morbid synovectomy technique.

The inflamed synovium of rheumatoid arthritis mimics neoplastic tissue [18]. The hypermetabolic activity and extensive neovascularity of rheumatoid synovium suggest an affinity for porphyrin-based hydrophobic photosensitizer compounds [17]. The quiescent nature of the normal articular tissues including articular cartilage suggests preferential retention of the compound in diseased tissue may be achieved with systemic administration of photosensitizing compounds. The spatial confinement of tissue within the lining of peripheral joints may make percutaneous or transcutaneous light delivery techniques possible.

We hypothesized that if PF were preferentially retained by the synovium in joints with active rheumatoid arthritis or other inflammatory arthritis, then photodynamic therapy might provide a suitable technique for selective destruction of synovium. Using rabbits with antigen-induced arthritis of knee joints, we undertook three sets of investigations. The objective of the first investigation was to characterize and quantify the time-dependent distribution of systemically administered PF within articular and periarticular tissues. The objectives of the second and third investigations were to evaluate photodynamic synovectomy (PDS) in the rabbit knee with respect to selectivity of tissue destruction and subsidence of inflammation using two different light delivery systems.

MATERIALS AND METHODS

Antigen-induced Arthritis Model

New Zealand White rabbits weighing 2–4 kg were sensitized over a 6-week period with two cutaneous injections of ovalbumin (Albumin, chicken egg, grade IV, Sigma, St. Louis, MO) suspended in Freunds adjuvant (1 ml of 10 mg/ml) (Sigma). Six weeks later, all knee joints were challenged with an inta-articular injection of 2.5 mg ovalbumin in 0.25 ml sterile saline solution. A monoarticular synovitis is then produced in 3–5 days [19,20].

Photosensitizer Preparation

For all experiments, PF (Quadra Logic Technologies, Vancouver, BC, Canada) provided as 75 mg of lyophillized powder, was reconstituted with 30 ml D5W solution to give a 2.5 mg PF/ml solution. Appropriate dosages based on weight were injected into ear veins with 25 gauge needles.

Biodistribution

Thirty New Zealand White rabbits were sensitized according to the antigen-induced arthritis protocol. One week after joint challenge, 25 animals received systemic intravenous injections with PF (2.0 mg/kg). Five animals received no injection and served as controls. Animals were killed at 6, 12, 24, 48, and 72 hours post-PF injection. Tissue samples were obtained of skin, quadriceps muscle, knee synovium, and articular cartilage. Wet tissue weight was measured (Mettler AE 163 balance) and tissue samples were frozen at −90°C (Revco Ultra Low Freezer) until dye extraction.

For extraction, tissue samples were homogeized (Homogenizer, Model PT 10/35, Brinkman Instruments, Westbury, NY) in 8 cc 0.1 N NaOH solution. Each sample was centrifuged at 17,000 RPM (Sorvall RC-5B, refrigerated superspeed centrifuge, DuPont instruments) for 20 minutes. The fluorescence of 2 cc of supernatant was measured with a spectrofluorimeter (Model Fluorolog 2, Spex Industries, Edison, NJ) and compared to that of standard solutions of PF with known concentration and mixed with supernatant of extracted control tissue. The excitation wavelength was 506 nm and a fluorescence spectrum between 600–700 nm was analysed. Untreated animals served as controls and for background correction.

The biodistribution of PF within synovium, articular cartilage, and meniscus was also studied

by fluorescence microscopy. At each of the harvest intervals of 6, 12, 24, 48, and 72 hours postinjection, tissue specimens were obtained from experimental and control animals. Samples were placed in O.C.T. medium and frozen at −90°C. Unstained frozen sections at 10 μm were prepared using a cryogenic microtome (Microm, Heidelberg, Germany) and analysed with a fluorescence microscope (Axiophot, Zeiss, Oberkochen, Germany) under epiluminescence of a mercury lamp (HBO 200 W/2, Zeiss). The excitation wavelength was between 300–500 nm; the emission above 580 nm was displayed on a video screen after light amplification in a silicon intensified tube video camera (MTI-DAGE SIT 66) and averaging of 60 images. The images were digitized and stored using a Macintosh II computer equipped with a digitizing board (DT 2255, Data Translation, Marlboro, MA). Frozen sections were compared to histological slides of the same area after staining with hematoxylin and eosin (H & E). Untreated animals served as controls.

Photodynamic Therapy

In the first set of experiments, 24 New Zealand white rabbits weighing 3–4 kg were divided into two groups of 12 each. All animals underwent a 6-week sensitization according to the antigen-induced arthritis protocol. The knee joints of all animals were challenged at 60 days with intra-articular injections of 0.25 ml ovalbumin solution with a concentration of 2.5 mg/ml. Injections required sedation with rompum and ketamine. Six days postchallenge, eight animals in group A and eight animals in group B received systemic injections of 2 mg/kg of PF via 25 gauge needle into an ear vein. Forty-eight hours post-PF injection, all animals in both groups were sedated with rompum and ketamine. The right knee of all animals received light illumination; 630 nm wavelength light energy was transmitted via two 400 μm diameter bare cleaved optical fibers through 22 gauge angiocatheters placed into the anteromedial and anterolateral compartments of the right knees (Fig. 1). A total light energy of ~100J/cm^2 was delivered to each joint at a power of 250 mW for 20 minutes. The left knees served as controls receiving angiocatheters without optical fibers or light energy delivery. After completion of treatment, animals were returned to cages and allowed unrestricted activity. One anesthetic death occurred in group A and and two deaths in group B.

The animals in Group A were sacrificed at 2 weeks, and the animals in Group B at 4 weeks. The stifle (knee) joints were dissected, the patellar tendon was transected, and the patella reflected laterally. The joint was examined grossly and a sample of synovium was retrieved from the infrapatellar fat pad and placed in 10% neutral-buffered formalin (Fisher Scientific, Pittsburgh, PA) for 1 week. The synovium was processed for glycol methacrylate embedment, cut into 3–5 μm sections and stained with H&E (Sigma). The remaining stifle joint was placed in 10% neutral-buffered formalin 3–4 weeks, split sagittally, and decalcified in 10% formic acid in sodium citrate buffer (Mallinckrodt, Paris, KY) for 6–8 weeks. The medial and lateral specimens were embedded in paraffin (Paraplast, VWR Scientific, Boston, MA) and 5–7-μm-thick serial sections were cut and stained with H&E or Safranin O for proteoglycan content. Three control samples from Group B were lost during histologic processing due to inadequate decalcification and inability to section. Gross observations at the time of harvest were recorded. Histologic sections were evaluated by a board certified pathologist (RGE) who was unaware of the treatment conditions.

In the second set of experiments, 30 animals were sensitized as described above. Twenty animals received systemic injections with PF (2 mg/kg). Light activation of the right knee of all animals was performed 48 hours postinjection with a single 600 μm diameter diffusion tip fiber (PDT systems, Santa Barbara, CA) placed into the anterior knee joint via an 18 gauge angiocatheter. A power of 200 mW was transmitted for 20 minutes. The remainder of the experimenter replicated the protocol described with animals sacrificed at 2 and 4 weeks.

For both treatments, histologic grading was performed on a scale of one (few cells or little effect) to four (numerous cells or large effect) for each of nine criteria. Samples were assessed for: (1) synovial necrosis, (2) synovial thickness, (3) plasma cell infiltrate, (4) macrophage infiltrate, (5) lymphocytic infiltrate, (6) neutrophil infiltrate, (7) cartilage integrity, (8) appearance of bone, and (9) appearance of articular cartilage. The anterior and posterior capsule of each knee was evaluated for each of the nine variables. Necrosis was rated as present or absent, whereas the other eight variables were rated on a scale of 0–4+, i.e., 0 = none, 1+ = mild, 2–3+ = moderate, 4 = severe.

Statistical analysis of the ranked data was performed with Kruskal-Wallis ANOVA analysis by ranks for the anterior section comparing right

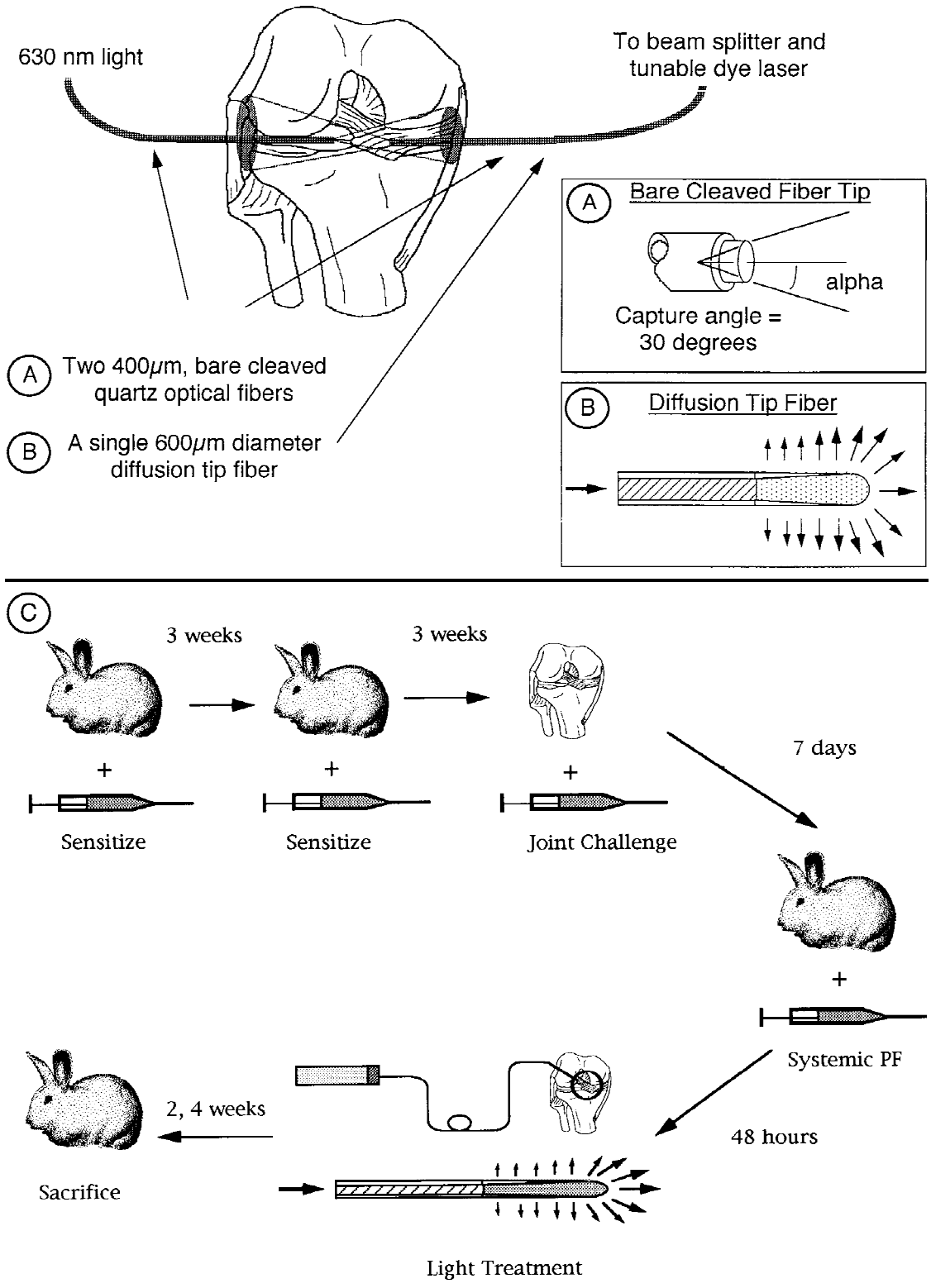


Fig. 1. Schematic of the treatment algorithm and the two light delivery systems used for the two rabbit treatment studies: **A.** First treatment study light delivery: two bare cleaved 400 μm quartz optical fibers placed via angiocatheters into the anteromedial and anterolateral rabbit knee joint. **B.** Second treatment study light delivery: a single 600 μm diameter diffusion tip fiber placed percutaneously in the anterior knee joint. **C.** Treatment algorithm for the two treatment studies using the antigen-induced arthritis rabbit model. Animals were sensitized with subcutaneous injections of ovalbumin in Freunds adjuvant at 0 and 3 weeks. At 6 weeks, the joints were challenged with intra-articular injections of ovalbumin solution. Immunologic response to the antigen produces intense inflammation and synovitis, mimicking an acute rheumatoid flare. Seven days later, as the synovium becomes more dense and proliferative, the animals receive systemic injections of BPD (2 mg/kg) via an ear vein. Three hours later, after the photosensitizer has distributed within the synovium, light is applied to the joint percutaneously, with either diffusion tip or bare cleaved optical fibers. At 2 and 4 weeks posttreatment, animals are sacrificed and knees are harvested for analysis.

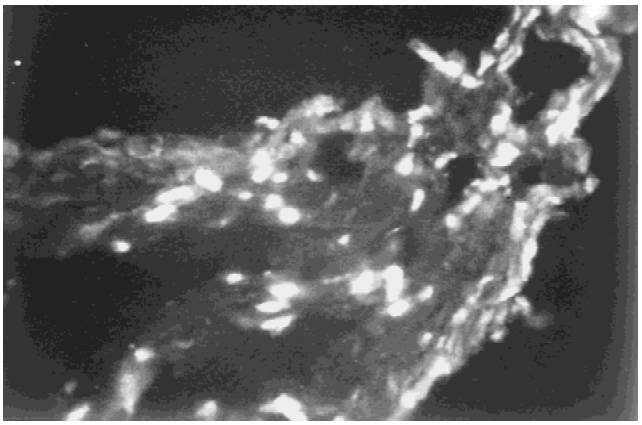


Fig. 2. Fluorescence photomicrograph: Synovium. (200×) PF fluorescence within synovial tissues 24 hours after a 2 mg/kg systemic injection. Note the diffuse cellular and extracellular photosensitizer distribution in the inflamed synovium.

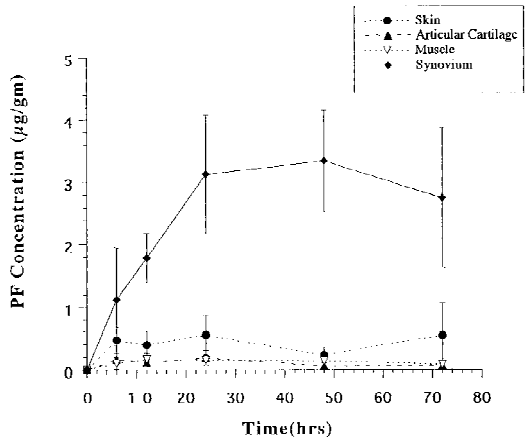
and left knees of both control and treatment groups for each parameter, followed by Dunn's follow-up for pairwise comparison. Wilcoxon signed rank testing compared anterior vs. posterior joint variables.

RESULTS

Biodistribution Study

Light and fluorescence microscopy. Consistent inflammation of synovium was seen in all animals at the time of sacrifice. PF fluorescence was not observed in articular cartilage or meniscus in either experimental or control animals at any time point following injection. Significant synovial uptake was observed at 12, 24, 48, and 72 hours following PF injection. PF fluorescence demonstrated a diffuse cellular and extracellular biodistribution at all time points (Fig. 2). No fluorescence was observed in synovium of control animals.

Extraction analysis. Quantitative spectroscopic analysis of extracted tissue samples demonstrated significant synovial uptake of the PF at all time points. Maximum uptake occurred 48 hours postinjection. Comparison with spectroscopic analysis of known sample concentrations revealed peak PF concentrations of 3.32 mcg/gm tissue at 48 hours. The concentration is comparable to therapeutic levels documented in successfully treated neoplastic tissues. Skin values between 0.228 and 0.539 mcg/gm were observed. Fluorescence levels in articular cartilage, and muscle were negligible and did not exceed baseline levels for endogenous porphyrin production (Fig. 3).



Biodistribution Study: Photofrin Extraction Values

Hours	Skin	Skin stdev	Art Cart	AC stdev	Muscle	Musc stdev	Synovium	Syn stdev
6	**0.470**	0.210	**0.162**	0.220	**0.101**	0.091	**1.122**	0.831
12	**0.394**	0.219	**0.114**	0.027	**0.164**	0.096	**1.785**	0.393
24	**0.548**	0.321	**0.189**	0.113	**0.150**	0.074	**3.121**	0.953
48	**0.229**	0.124	**0.058**	0.049	**0.133**	0.091	**3.332**	0.815
72	**0.539**	0.521	**0.057**	0.043	**0.078**	0.049	**2.741**	1.127

Tissue values of PF (μg/gm tissue) following a 2 mg/kg systemic PF injection in a rabbit antigen induced arthritis model.

Fig. 3. Biodistribution study: extraction data. Concentration of PF in synovium and control tissues following a 2 mg/kg systemic PF injection.

Treatment Results

Bare cleaved fiber irradiation. On gross observation, the right knees of all treated animals demonstrated edema and sensitivity to exam for 3–5 days after irradiation. Sensitivity to palpation subsided by 5 days in treated knees but continued in the control knees throughout the duration of the study.

Although all animals developed a synovitis, the degree of inflammation as graded by histology from anterior knee joint samples was variable and ranged from a slight inflammatory response (score = 1) to highly inflamed (score 3.5). Histology of anterior synovial samples from treated animals with an inflammatory score of at least 2 on the left side did not demonstrate a significant decrease in inflammation on the right side relative to the left side. There was no significant differences between right and left knee scores in the control animals. One necrotic synovial specimen was obtained from the anterior knee joint of a treatment knee 2 weeks posttreatment. The ne-

TABLE 1. Knees Showing Synovial Necrosis*

	Bare cleaved fibers		Diffusion tip	
Time after treatment	2 weeks	4 weeks	2 weeks	4 weeks
Anterior joint	1/6 (17)	0/6 (0)	3/7 (43)	3/8 (38)
Posterior joint	0/6 (0)	0/6 (0)	0/7 (0)	0/8 (0)

*Presence of synovial necrosis in histologic cross sections of rabbit antigen induced arthritis knees obtained two and four weeks post treatment with two different modes of light delivery. Data are expressed as the number of necrotic specimens in the knees of treatment animals over the total number of specimens for the treatment group. () represent % of animals exhibiting necrosis. No necrosis was observed in any control specimen.

TABLE 2. Plasma Cell Infiltrate: Histologic Evaluation*

A. Two weeks posttreatment

Sample	Cleaved fiber	Diffusion tip
TREATED		
R-anterior	0.83 ± 1.37	0.50 ± 0.30
R-posterior	2.50 ± 0.33	2.17 ± 0.17
L-anterior	1.75 ± 0.67	1.67 ± 1.07
L-posterior	2.42 ± 0.33	2.00 ± 0.00
CONTROL		
R-anterior	0.50 ± 0.50	2.00 ± 1.00
R-posterior	2.50 ± 0.50	3.00 ± 1.00
L-anterior	1.75 ± 0.92	2.33 ± 0.33
L-posterior	2.50 ± 0.33	2.33 ± 0.33

B. Four weeks posttreatment

Sample	Cleaved fiber	Diffusion tip
TREATED		
R-anterior	1.60 ± 0.33	1.25 ± 0.79
R-posterior	2.60 ± 0.33	2.50 ± 0.29
L-anterior	2.17 ± 0.57	1.40 ± 0.80
L-posterior	2.17 ± 0.17	2.40 ± 0.30
CONTROL		
R-anterior	1.25 ± 0.25	2.00 ± 0.71
R-posterior	2.25 ± 0.25	2.50 ± 0.25
L-anterior	2.00 ± 0.67	2.00 ± 0.00
L-posterior	2.50 ± 0.33	3.00 ± 0.67

*All samples were graded on a scale of 0 (no infiltrate) to 4 (severe infiltrate). Values are expressed ± variance. All right knees (R) received light treatment and left knees (L) received no light. Treated animals received photosensitizer (PS) injections; control animals received no PS.

crosis was localized to the site of light delivery in the region of the anterior fat pad. No necrosis was observed in any control specimens. The necrosis data from gross and histologic observations are summarized in Table 1.

Evaluation of the joint sagittal sections revealed no additional necrosis of synovium in either treatment or control animals. Articular cartilage appeared well preserved with no ultrastructural changes. Diminished proteoglycan content as determined by safranin O staining was observed uniformly in all treatment and control specimens. At both 2 and 4 weeks, anterior synovial plasma cell infiltrate was significantly reduced relative to control knees that received chemical without light, or no chemical or light ($P < 0.05$). Significant differences were not observed at 2 or 4 weeks relative to knees receiving light without photosensitizer (Table 2).

Diffusion tip fiber irradiation. When a diffusion tip fiber was used for light delivery, gross observations similar to those noted for bared cleaved fiber irradiation were recorded posttreatment. No erythema developed over anterior knee joints. Knee joint sensitivity to exam occurred transiently for 3–5 days in treatment knees while persisting in the controls.

Anterior synovial necrosis as recorded on sagittal sections of the entire knee joint was observed in 43% of treatment specimens at 2 weeks and 38% of specimens at 4 weeks. Necrosis was confined to the region of light activation and was characterized by loss of cellularity and structure with an eosinophilic matrix and scattered pyknotic nuclei (Fig. 5). No necrosis of articular cartilage or periarticular tissues was observed in any specimen (Fig. 6). Proteoglycan staining with safranin O was also uniformly decreased in the articular surfaces of all treatment and control specimens.

Plasma cell infiltrate as judged on a scale of zero (none) to four (extensive) was significantly reduced in the anterior synovial treatment specimens at 2 weeks (ANOVA $P < 0.01$) with significant decrease by paired *t*-test analysis relative to animals receiving chemical and no light, or no chemical or light. A significant reduction in synovial infiltrate relative to all controls was observed at 2 weeks when noninflamed animals were excluded. By 4 weeks, the reduction in plasma cell infiltrate was no longer significant. No significant reduction in macrophage, lymphocyte of neutrophil infiltrates were observed in the treatment synovium in either study. No deleterious effect or structural change was observed in any articular cartilage specimen (Table 2).

Synovial hyperplasia (Fig. 4) revealed a similar pattern. Hyperplasia was significantly reduced in the anterior synovial treatment specimens at 2 weeks (ANOVA $P < 0.01$) with significant decrease by paired t-test analysis relative to animials receiving chemical and no light, or no

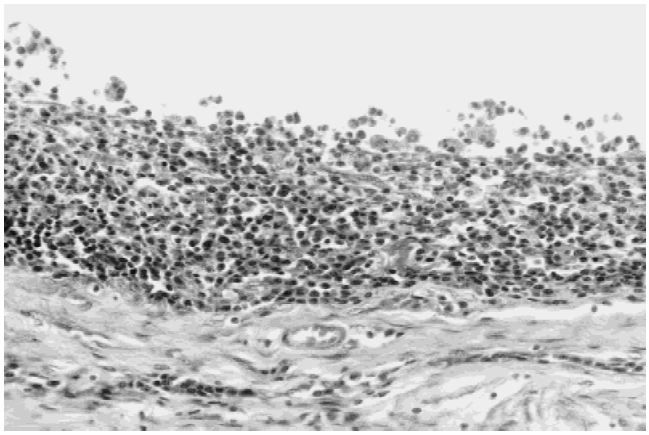


Fig. 4. Synovial hyperplasia: (200×). Photomicrograph of a full thickness section of posterior synovium with marked increased thickness and a dense infiltrate of lymphocytes and plasma cells. Section was obtained 3 weeks after joint challenge with ovalbumin solution in an untreated knee (H&E stain).

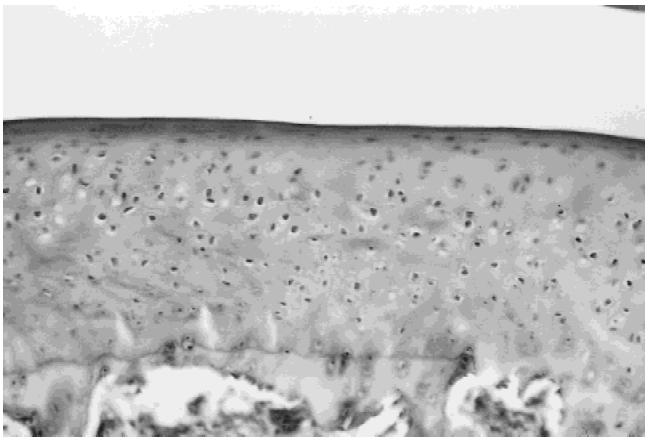


Fig. 6. Articular cartilage: (200×). Full thickness section of unaffected articular cartilage obtained from the anterior femoral condyle of the rabbit knee. Section was obtained from the region of light activation, 4 weeks after photodynamic treatment (H&E stain).

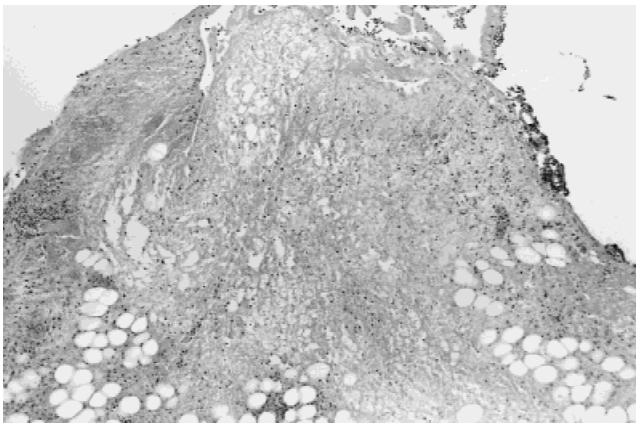


Fig. 5. Synovial necrosis: (100×). Necrotic anterior synovium characterized by loss of cellular detail, amorphous eosinophilic debris and pyknotic nuclear fragments; 2 mm diameter necrotic section was obtained in the region of the anterior fat pad of a treated rabbit knee (H&E stain).

TABLE 3. Synovial Hyperplasia: Histologic Evaluation

Diffusion Tip Study*

Sample	2 weeks	4 weeks
TREATED		
R-anterior	0.87 ± 0.50	1.25 ± 0.79
R-posterior	2.00 ± 0.17	2.50 ± 0.29
L-anterior	2.00 ± 0.40	1.40 ± 0.80
L-posterior	2.00 ± 0.00	2.40 ± 0.30
CONTROL		
R-anterior	2.33 ± 2.33	2.00 ± 0.71
R-posterior	3.67 ± 0.33	2.50 ± 0.25
L-anterior	2.67 ± 0.33	2.00 ± 0.00
L-posterior	2.67 ± 0.33	3.00 ± 0.67

*All samples were graded on a scale of 0 (no infiltrate) to 4 (severe infiltrate). Values are expressed ± variance. Right knees (R) of all animals received light treatment and to the anterior portion of the joint. Left knees (L) received no light. Treated animals received photosensitizer (PS) injections; control animals received no PS.

chemical or light. No significant decrease was observed relative to specimens receiving light only ($P = 0.10$). A similar pattern was observed at 4 weeks (ANOVA $P < 0.003$); however, paired analysis demonstrated significance of treatment knees only relative to controls receiving no light or chemical (Table 3). If noninflamed animals were removed from analysis, synovial hyperplasia was significantly reduced at 2 weeks in treatment synovium relative to all control groups (ANOVA $P < 0.0001$, $P < 0.05$ for paired analysis of treated right knees relative to controls).

DISCUSSION

Numerous chemical synovectomy procedures have been investigated previously for the destruction of synovium in patients with rheumatoid arthritis refractory to standard medical treatment. These procedures have been abandoned for their high associated morbidity and for their inability to selectively destroy the diseased synovium. Currently, researchers have achieved success destroying rheumatoid synovium with intra-articular injections of radioactive compounds [13,14]. Widespread use of this technique may be

limited by the required handling of radioactive compounds. Hip joint use may also be contraindicated by reproductive organ exposure to radioactivity.

Our findings of selective uptake of PF by inflamed synovium compared to other articular tissues, and destruction of this synovium following illumination, suggest that the concept of photodynamic synovectomy using a photo-activatable compound has clinical promise. Before the technique can be applied clinically, however, there are a number of issues that require further experimental investigation. The clinician has six principal variables that can be manipulated to control the photochemical synovectomy: choice of photoactivated compound, dose of the compound, route of drug administration, and timing, dose, and techniques of illumination.

Forty-eight hours after injection of the 2 mg/kg dose, the concentration of PF that we observed in the inflamed synovium (3.32 μg/g) was within the therapeutic range of 2–8 mcg/gm determined in studies of antineoplastic therapies. Concentrations in the skin at this time, although only about one-third of the synovial concentration, were still sufficient to warrant protection of the patient from light so as to avoid phototoxic side effects. Current clinical trials with PF protect patients from sunlight exposure for 4–6 weeks.

The selection of the appropriate time to activate the PF is based on the preferential retention in pathologic tissue with gradual clearance of the material from all normal tissues. Ideally, there is a critical period in which adequately toxic levels of the compound are present in inflamed synovium, whereas levels in adjacent tissues are low enough to minimize toxicity should they be illuminated. We found 48 hours postinjection to be a suitable time for illumination.

When activating of PF with light, the surgeon can control the power of light delivered, its duration, and its orientation, although scattering of light within the joint may activate the compound in some tissues outside the target area. We used a light power of 250 mW at the source. In order to achieve the intended dose of 100 J/cm^2 an exposure time of ~20 minutes was required. Delivering light at a higher power is not practical as the light beam itself can cause thermal damage, and overexposure of the PF may cause photobleaching and inactivation [21]. The bare cleaved optical fibers we used to illuminated the joints in the first set of experiments were far from ideal and allowed little control over orientation and uniformity of illumination. The fibers provided a narrow region of effective photosensitizer activation as confirmed by the lack of necrosis in most treatment specimens and the small zone of necrosis observed histologically in the one necrotic specimen. More sophisticated diffusion tip fibers in the second treatment experiment provided broader, more uniform activation in the region of interest but also remained suboptimal, providing little light outside the region of the fat pad and intercondylar notch. The improved results in the second treatment group confirms the importance of the mode of light activation in providing an effectiv photochemical treatment. In both experiments, necrosis was observed only in the region of light delivery confirming the selectivity of this treatment modality.

Despite the likelihood that articular cartilage would be illuminated during activation of the PF, the cartilage appears to suffer no damage. Its low absorbance of systemically administered PF is probably due to the relative avascularity and low cellularity of cartilage. Moreover, the low level of oxygen within cartilage should suppress the destructive effects of activated PF, which depends on oxidative reactions for its catabolic effects. Articular cartilage appeared well preserved in both the treated and control joints. No fraying, necrosis, chondrocyte hyperplasia, or degeneration was observed, and Safranin-O staining was similar in treated and untreated joints. The lack of safranin O staining of articular cartilage in all specimens suggests leaching of proteoglycans by the prolonged decalcification process. Staining of growth plates was seen with safranin O in these immature animals.

Results of the treatment with diffusion tip fibers suggest that selective destruction of inflamed synovium can be achieved by systemic administration of PF followed by intra-articular light activation. Gross and microscopic observations confirmed focal, sharply defined synovial necrosis and decreased synovial thickness at 2 and 4 weeks postillumination. The clinical observation of transient knee joint sensitivity to palpation suggests that the treatment and resulting necrosis produce additional inflammation that abates by 5–7 days posttreatment. Necrosis was evident in 43% and 38% of anterior synovial treatment specimens at 2 and 4 weeks, respectively, with a statistically significant decrease in the amount of synovial hyperplasia and plasma cell infiltrate at 2 weeks only. The lack of uniformity in clinical outcome may reflect variations and inefficiencies

in light delivery, nonuniform photosensitizer distribution as well as the persistence of the antigenic challenge in this progressive model of rheumatoid arthritis.

In the clinical setting, photodynamic synovectomy most likely will develop in two forms, depending on the size of the treated joints. For larger joints such as the knee or shoulder, percutaneous light delivery techniques will be used for the activation of systemically administered or locally injected photosensitizer. The light techniques may include use of optical fibers delivered via small gauge needles, or via use of arthroscopic instrumentation. For small or multiloculated joints, as in the hand or wrist, transdermal light activation techniques will broadly activate a systemically administered photosensitizer that localizes diffusely to inflamed articular and periarticular tissues.

The production of necrosis by the minimally invasive or noninvasive photodynamic synovectomy techniques may mandate use of supplemental therapies. A large load of necrotic tissue in a confined joint space may induce secondary inflammation. The inflammation and periarticular edema if coupled with joint immobility due to patient discomfort may lead to periarticular fibrosis. Joint irrigation may be required at a delayed time point to remove necrotic debris. As with radiation synovectomy, secondary inflammation also may be controlled through use of steroids delivered locally to the joints. Production of necrosis in the inflamed tenosynovium of rheumatoid hands may present a more difficult challenge if the necrosis induces scarring or fibrosis along tendon sheaths. Future transcutaneous animal studies will address these issues.

In summary, these studies suggest that photodynamic synovectomy may be effective as a means of ablating diseased synovium in medically refractory cases of inflammatory arthritis by the intra-articular light activation of the systemically administered photosensitizing compound PF. The photoactive compound appeared to be rapidly cleared from adjacent tissues, and we observed no deleterious effects in periarticular structures. Optimization of light delivery and other photochemical parameters are clearly essential for effective development of this new treatment modality. Should experimental treatments prove successful, photochemical synovectomy may prove to be a less invasive and less morbid therapeutic alternative for patients suffering from rheumatoid arthritis.

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